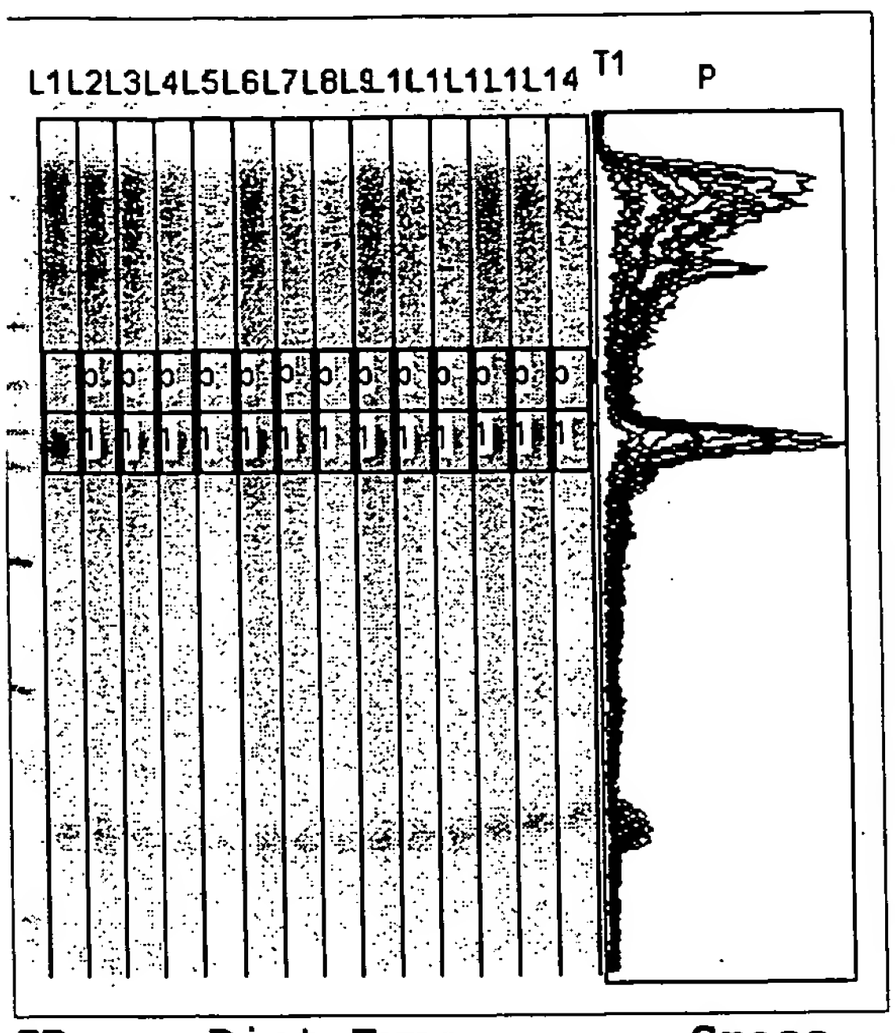
DOCUMENT I

INHIBITION OF HIV AND HSV TRANSCRIPTION BY METHYLATED NOGA

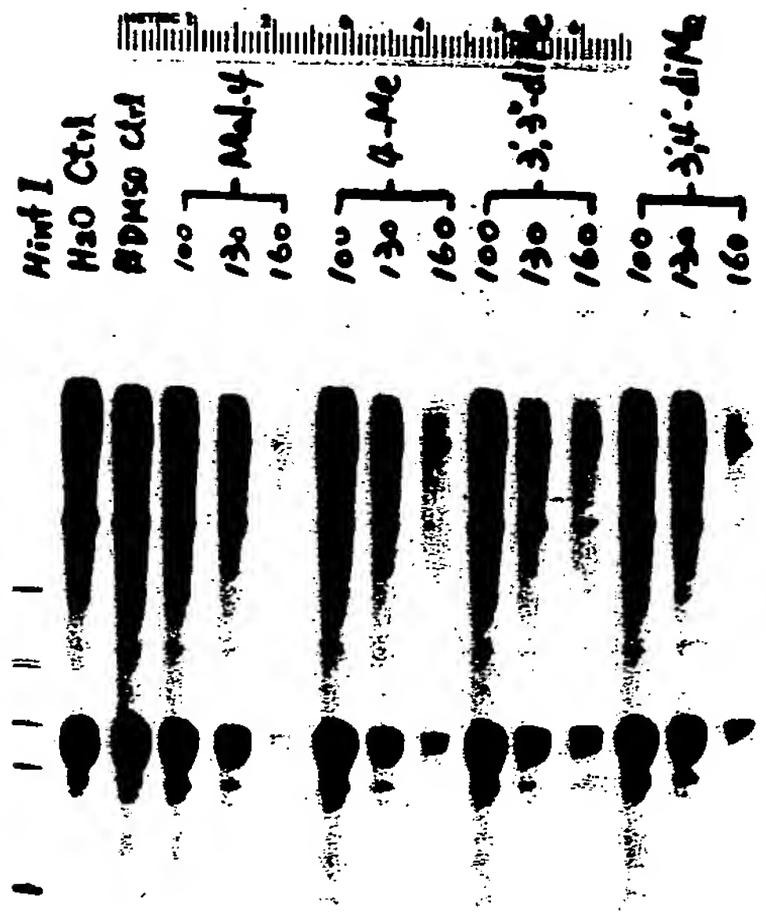
WORK WAS ACCOMPLISHED IN THE FIRST 6 MONTHS OF 1996 IN ROOM 249 LEVI BUILDING, JOHNS HOPKINS UNIVERSITY, INUENTORS LAB.

File:012696.IMG Acquired:1/29/96 11
huang
Bitmap Display Range:0-63 counts, 1

#T1 Lanes
Background Subtraction: regions

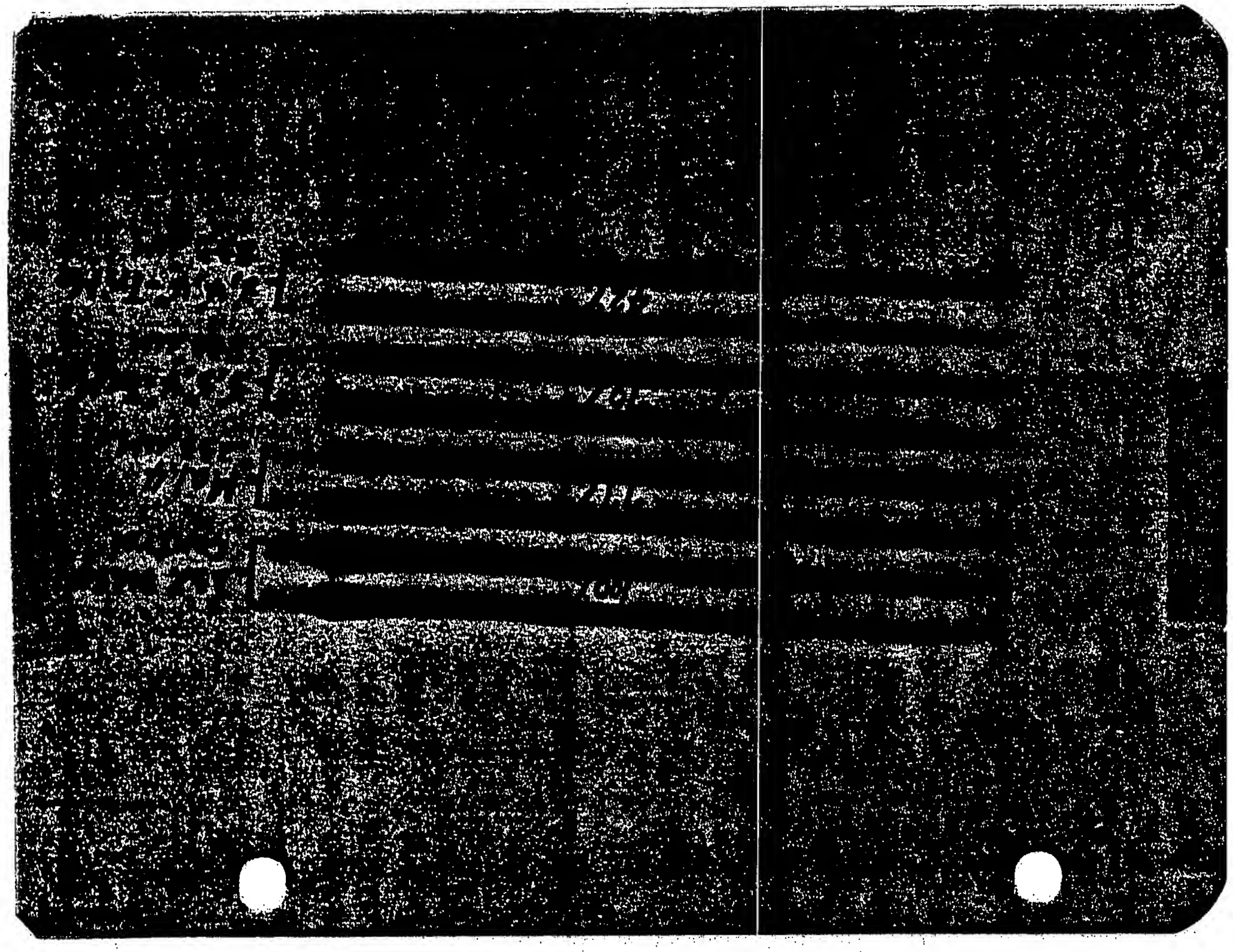


ID	Dist (mm)	Type	Gross Counts		
Lane #1	**Bkgd subtr =12.62/sqmm**				
2	42.5	Bkgd	757		
1	53.0	Unkn	3,631		
Lane #2	**Bkgd subtr =19.38/sqmm**				
2	42.5	Bkgd	1,163		
1	53.0	Unkn	3,464		
Lane #3	**Bkgd subtr =16.07/sqmm**				
2	42.5	Bkgd	964		
1	53.0	Unkn	2,832		
Lane #4	**Bkgd subtr =12.20/sqmm**				
2	42.5	Bkgd	732		
1	53.0	Unkn	1,936		
Lane #5	**Bkgd subtr =6.13/sqmm**				
2	42.5	Bkgd	368		
1	53.0	Unkn	635	368	267
Lane #6	**Bkgd subtr =17.23/sqmm**				
2	42.0	Bkgd	1,034		
1	53.0	Unkn	3,575	1,034	2,541
Lane #7	**Bkgd subtr =11.30/sqmm**				
2	42.5	Bkgd	678		
1	53.0	Unkn	1,764	678	1,086
Lane #8	**Bkgd subtr =8.08/sqmm**				
2	42.5	Bkgd	485		
1	53.0	Unkn	821	485	336
Lane #9	**Bkgd subtr =16.23/sqmm**				
2	42.5	Bkgd	974		
1	53.0	Unkn	3,012	974	2,038
Lane #10	**Bkgd subtr =10.90/sqmm**				
2	42.5	Bkgd	654		
1	53.0	Unkn	1,680	654	1,026
Lane #11	**Bkgd subtr =8.38/sqmm**				
2	42.5	Bkgd	503		
1	52.5	Unkn	1,241	503	738



ID	Dist (mm)	Type	Gross Counts	Bkgd Subtr	Net Total
Lane #1	**Bkgd subtr =12.62/sqmm**				
2	42.5	Bkgd	757		
1	53.0	Unkn	3,631	757	2,874
Lane #2	**Bkgd subtr =19.38/sqmm**				
2	42.5	Bkgd	1,163		
1	53.0	Unkn	3,464	1,163	2,301
Lane #3	**Bkgd subtr =16.07/sqmm**				
2	42.5	Bkgd	964		
1	53.0	Unkn	2,832	964	1,868
Lane #4	**Bkgd subtr =12.20/sqmm**				
2	42.5	Bkgd	732		
1	53.0	Unkn	1,936	732	1,204
Lane #5	**Bkgd subtr =6.13/sqmm**				
2	42.5	Bkgd	368		
1	53.0	Unkn	635	368	267
Lane #6	**Bkgd subtr =17.23/sqmm**				
2	42.0	Bkgd	1,034		
1	53.0	Unkn	3,575	1,034	2,541
Lane #7	**Bkgd subtr =11.30/sqmm**				
2	42.5	Bkgd	678		
1	53.0	Unkn	1,764	678	1,086
Lane #8	**Bkgd subtr =8.08/sqmm**				
2	42.5	Bkgd	485		
1	53.0	Unkn	821	485	336
Lane #9	**Bkgd subtr =16.23/sqmm**				
2	42.5	Bkgd	974		
1	53.0	Unkn	3,012	974	2,038
Lane #10	**Bkgd subtr =10.90/sqmm**				
2	42.5	Bkgd	654		
1	53.0	Unkn	1,680	654	1,026
Lane #11	**Bkgd subtr =8.38/sqmm**				
2	42.5	Bkgd	503		
1	52.5	Unkn	1,241	503	738

Lane #12		**Bkgd subtr =15.18/sqmm**				
2	42.5	Bkgd	911			
1	52.5	Unkn	3,114	911	2,203	
Lane #13		**Bkgd subtr =11.92/sqmm**				
2	42.5	Bkgd	715			
1	52.5	Unkn	2,438	715	1,723	
Lane #14		**Bkgd subtr =6.07/sqmm**				
2	42.5	Bkgd	364			
1	52.0	Unkn	930	364	566	



2-29-96 Test of HSV - pBR 322 Δ 380 template inhibition by Mal 4

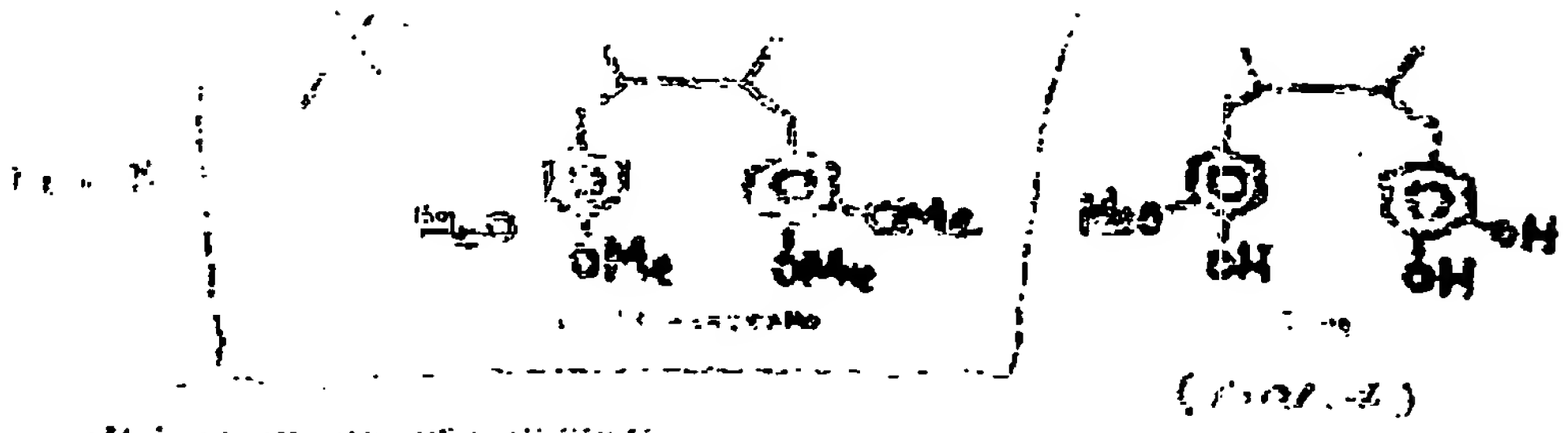
Will use 300 nanogram template in 12.8 λ. Will do Marley at 60% conc. at 160, 190 and 220 ng/rxl Mal 4.

1. DMSO cntrl .4 λ
2. Mal 4 160
3. 190
4. 220

Dissolve DNA in 9.6 λ H_2O. Add. 4 λ Mal 4 dilutions and incubate 15 min at 30°. For 60% Marley need 4.5 λ extract + 3 λ buffer.

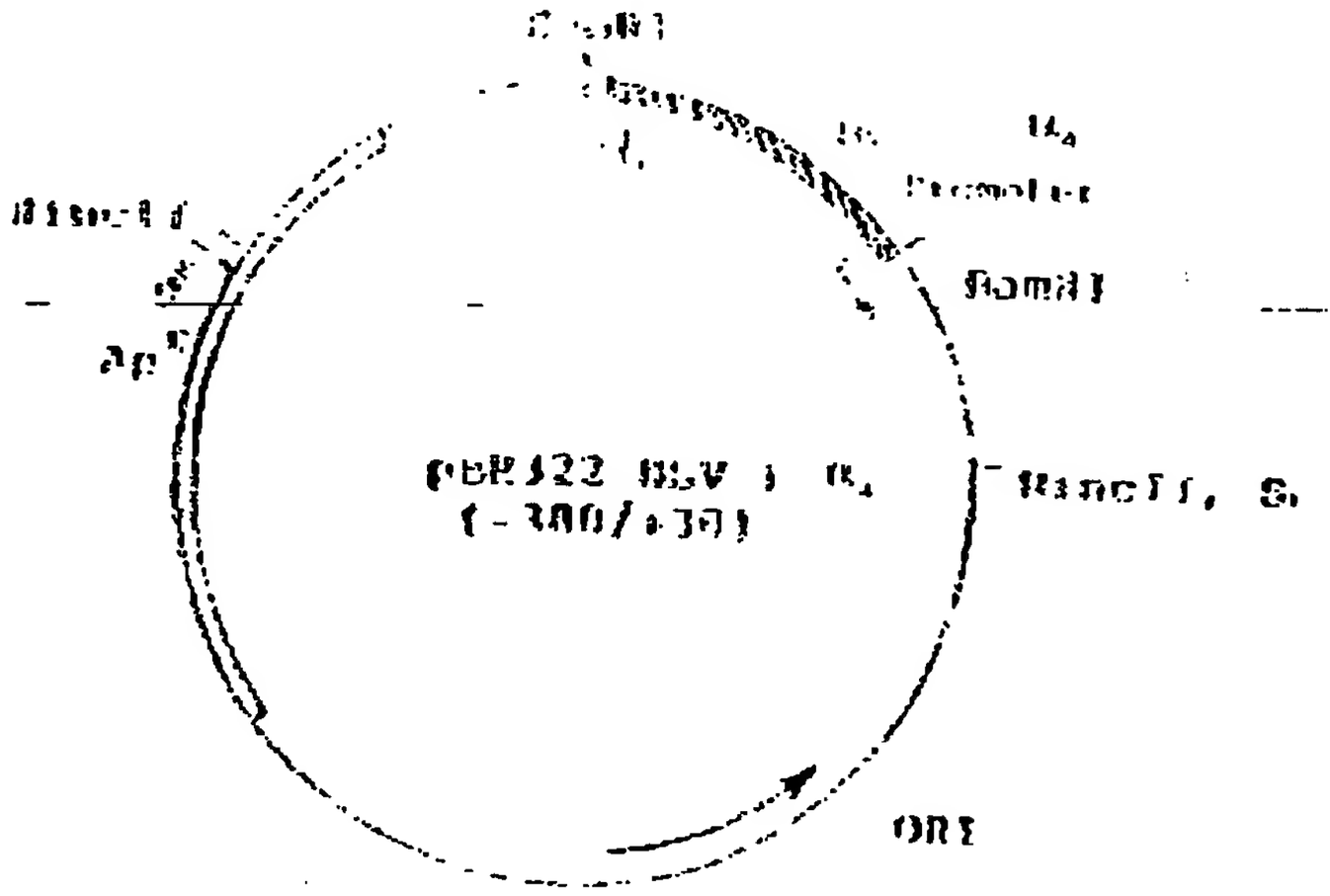
t using 50% extract. Loaded ½ Ex.

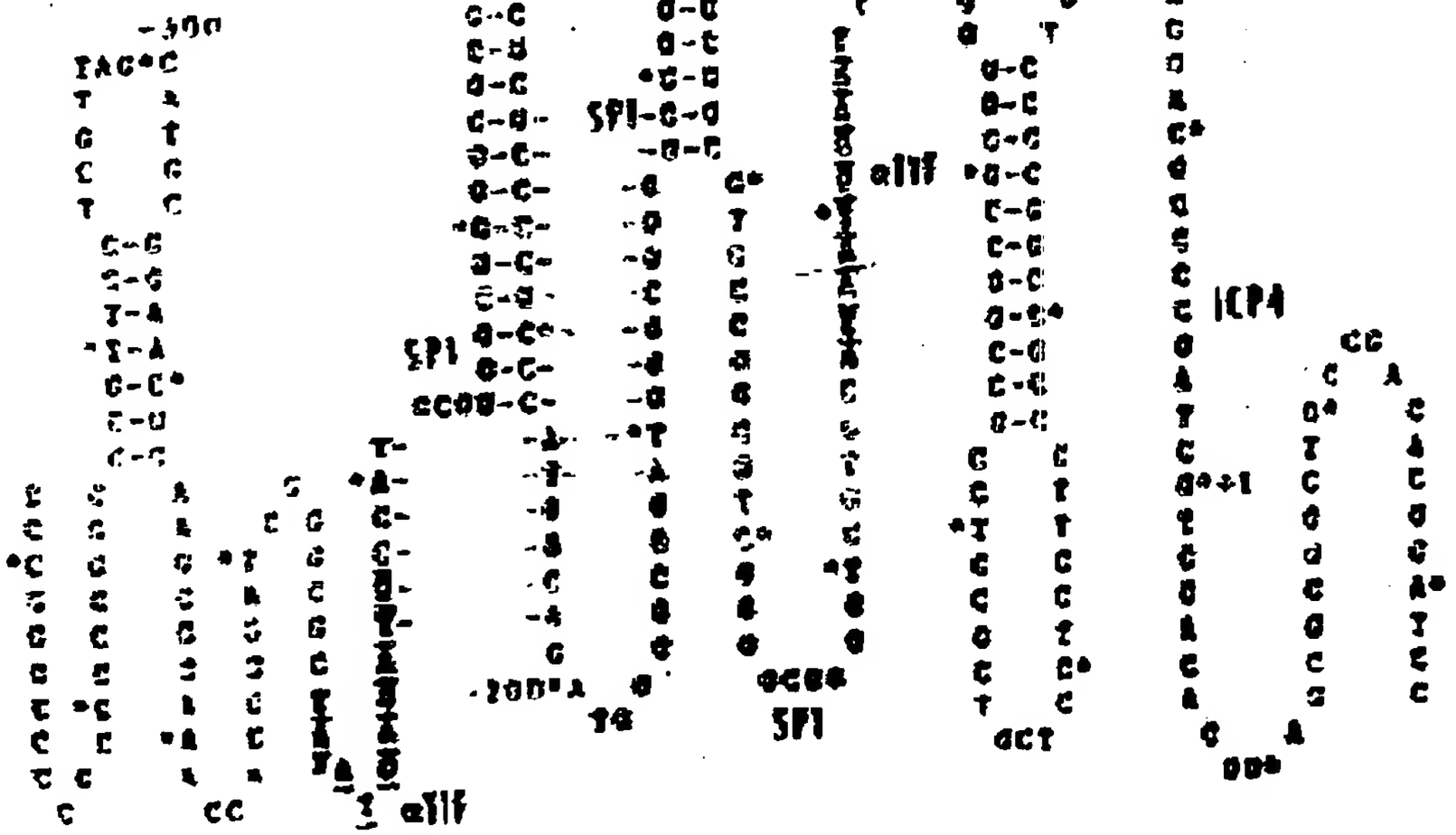
the 160, 190, 220 Mal 4.



(Prior Art)

B. Template of HSV t_{α_4}





C. Effect of Mal 4 on the promoter activity of α_4 gene *in vitro*

Template	% Transcription (Mal 4 µg/rxn)				
	0	100	130	160	190
pBR322 HSV 1 t_{α_4}	100	20	0	6	0

(Ref. 52. Mackem, S. & Roizman, B.

DOCUMENT II

A. DESIGN AND INSTRUCTION FROM DR. RUCHIH C. HUANG, INVENTOR, TO DR. H. S. CHEN FOR CARRYING OUT EXPERIMENTS USING TETRAMETHYL NDGA TO INHIBIT HSV IN VERO CELLS AND TOXICITY STUDIES OF THE DRUG IN MICE

LETTER DATED IN MAY 21 AND MAY 24, 1996

B. RESULTS RECEIVED BY DR. HUANG 6/25/96

(A)

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410 516-7330 FAX (410) 516-5213

DR. CHEN HONGSHAN
Fax 516-746-6618 NDGA 2mg/50ml DMSO. 于2005-7-?

[illegible]教授

① NDGA 与 MA1 4种配[illegible], 今日 FED EXPRESS 寄上 TETRAMETHYL
② 体外抑制 HIV integrase 的工作, NDGA 250mg 在一瓶 DMSO 溶液
③ NDGA对HIV-1 [illegible]細胞效果 [redacted] of HSV-1 動物實驗 [illegible]
每只, [illegible] Kg
DOSE: 2mg/[illegible] ([illegible])
每日一次, 共注射 5-7 天.
注射方式 [illegible] 是 I.V. [illegible]
至於 HSV-1 [illegible]
已決定, 又 [illegible]
定. TETRAMETHYL NDGA [illegible] (Sp) 之 TRANSCRIPTION [illegible] HSV-1 REPLICATION [illegible]
[illegible]
解釋 ([illegible] PROMOTER [illegible] SP₁ [illegible]).

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(410) 516-7330 / FAX (410) 516-5213

1996年5月24日

回傳一次。

DR. CHEN HONGSHAN
Fax 516-746-6615

[illegible]研究指導

今日 FED EXPRESS 寄上 TETRA METHYL NDGA 250ug 溶於 DMSO 中 供 INHIBITION OF HSU-1 動物實驗，老鼠 (MOUSE)
DOSE: 2ug/50ul (SC INOCULATION) 每日一次，共連續 [illegible] 天。
注射方式 [illegible]。
至於 HSU-1 infection 量 [illegible] 已決定，又如何 [illegible] 決定。TETRA METHYL NDGA 在 [illegible] (α_4) 之 TRANSCRIPTION 之一 [illegible]，HSU-1 REPLICATION [illegible] [illegible] 學術 [illegible] [illegible] [illegible] ([illegible]) [illegible] SP [illegible]。

要直接溶在100% DMSO中 每天/每兩天 二次 打 0.05ml 含 TETRA-methyl NDGA 2ug

II B

1996.6.25 給美國女士

C-4N Doc

Table 1. Cytotoxicity and inhibition of HSV-1 CPE by 4N in vero cell cultures

1996, 6-20-25

Agent	Conc. ug/ml	Cytotoxicity(OD570) X ± SD	Inhibition of cell growth %	TC50 ug/ml	HSV-1 CPE (OD570) X ± SD	Inhibition of HSV-1CPE %	IC50 ug/ml
4-N	1000.00	0.03, 0.03, 0.03, 0.02 0.03 ± 0.01	97.05	83	0.04, 0.05, 0.00, 0.00 0.02± 0.03	-	<0.98
	500.00	0.03, 0.04, 0.05, 0.04 0.04± 0.01	96.08		0.05, 0.11, 0.07, 0.04 0.07± 0.03	-	
	250.00	0.30, 0.30, 0.30, 0.30 0.3± 0	70.59		0.30, 0.26, 0.22, 0.28 0.27± 0.03	-	
	125.00	0.34, 0.38, 036, 0.39 0.37± 0.02	63.73		0.57, 0.45, 0.62, 0.60 0.56± 0.08	31.5	
	62.50	0.45, 0.46, 0.45, 0.45 0.45± 0.01	55.88		0.52, 0.49, 0.68, 0.66 0.59± 0.10	34.8	
	31.30	0.81, 0.80, 0.86, 0.85 0.83± 0.03	18.63		0.90, 0.84, 0.79, 0.87 0.85± 0.05	64	
	15.60	1.08, 1.02, 1.00, 0.98 1.02± 0.04	0		0.72, 0.92, 0.68, 0.92 0.81± 0.13	59.6	
	7.80	1.25, 1.04, 1.00, 1.11 1.10± 0.11	-0.08		0.74, 0.79, 0.96, 1.03 0.88± 0.14	67.4	
	3.90	1.10, 1.08, 1.06, 1.08 1.08± 0.02	-0.06		0.79, 1.07, 0.83, 0.76 0.86± 0.14	65.2	
	1.95	1.10, 1.20, 1.22, 1.00 1.10± 0.1	-0.08		0.78, 0.85, 0.81, 0.76 0.80± 0.04	58.4	
	0.98	1.30, 1.25, 1.15, 1.15 1.20± 0.08	-0.18		0.83, 0.73, 0.80, 0.83 0.80± 0.05	58.4	
	CC	1.02, 1.03, 1.00, 1.04 1.02± 0.02			1.13, 1.22, 1.18, 1.15 1.17± 0.04		
	vc X± SD				0.24, 0.29, 0.31, 0.26 0.28± 0.03		

Table 2. Inhibition of HSV-1 CPE by ACV on vero cell

1996, 6, 20-25.

Agent	CONC. ug/ml	HSV-1 CPE(OD570)	Inhibition %	IC50 ug/ml
ACV	1000 X± SD	0.70,0.53,0.57,0.62 0.61± 0.07	37	<0.98
	500 X± SD	0.63,0.38,0.62,0.49 0.53± 0.12	28.1	
	250 X± SD	0.50,0.49,0.64,0.87 0.63± 0.18	39.3	
	125 X± SD	0.41,0.43,0.53,0.66 0.51± 0.11	25.8	
	62.5 X± SD	0.73,0.72,0.73,0.76 0.74± 0.02	51.7	
	31.3 X± SD	0.93,0.90,0.86,0.93 0.91± 0.03	70.8	
	15.6 X± SD	1.00,0.99,0.98,1.02 1.00± 0.02	80.9	
	7.8 X± SD	1.09,1.11,1.16,1.10 1.12± 0.03	94.4	
	3.9 X± SD	1.13,1.07,1.10,1.09 1.10± 0.03	92.1	
	1.95 X± SD	1.14,1.12,1.17,1.11 1.14± 0.03	96.6	
	0.98 X± SD	0.76,0.80,0.82,0.92 0.83± 0.07	61.8	
	CC X± SD	1.13,1.22,1.18,1.15 1.17± 0.04		
	VC X± SD	0.24,0.29,0.31,0.26 0.28± 0.03		

①

医药生物技术研究所

4N 对 Vero细胞毒性　　96. 6. 25

药量 μg/ml	细胞OD值(570)	X±SD	破坏%	IC50
1000	0.03 0.03 0.03 0.02	0.03±0.01	97.05	83 μg/ml
500	0.03 0.04 0.05 0.04	0.04±0.01	96.08	
250	0.3 0.3 0.3 0.3	0.3±0	70.59	
125	0.34 0.38 0.36 0.39	0.37±0.02	63.73	
62.5	0.45 0.46 0.45 0.45	0.45±0.01	55.88	
31.3	0.81 0.80 0.86 0.85	0.83±0.03	18.63	
15.6	1.08 1.02 1.00 0.98	1.02±0.04	0	
7.8	1.25 1.04 1.00 1.11	1.1±0.11	-0.08	
3.9	1.10 1.08 1.06 1.08	1.08±0.02	-0.06	
1.95	1.10 1.20 1.22 1.00	1.1±0.1	-0.08	
0.98	1.30 1.25 1.15 1.15	1.2±0.08	-0.18	

CC 1.02 1.03 1.00
1.02±0.02

VC 0 0 0.0

L605

(2)

医药生物技术研究所

4N HSV-I 抑制实验 9.6.6.水

药量 μg/ml	OD值 (570 nm)				抑制率%	IC_{50} (μg/ml)
1000	0.04	0.01	0.00	0.00	92.86	< 0.98
	0.02±0.02					
500	0.05	0.11	0.07	0.04	75.50	
	0.07±0.03					
250	0.30	0.36	0.20	0.18	7.57	
	0.27±0.03					
125	0.57	0.45	0.62	0.60	31.5	
	0.56±0.08					
62.5	0.52	0.49	0.68	0.66	34.8	
	0.59±0.10					
31.3	0.90	0.84	0.79	0.87	64.00	
	0.85±0.05					
15.6	0.75	0.92	0.68	0.92	59.6	
	0.81±0.13					
7.8	0.74	0.79	0.96	1.03	67.4	
	0.88±0.14					
3.9	0.79	1.07	0.83	0.76	62.2	
	0.86±0.14					
1.95	0.78	0.85	0.81	0.76	58.4	
	0.80±0.04					
0.98	0.83	0.73	0.80	0.83	58.4	
	0.85±0.05					
CC	1.13	1.22	1.18	1.15		
	1.17±0.04					

VA L605 0.24 0.29 0.31 0.26

0.28±0.03

(3)

医药生物技术研究所

ACV对HSV-I作用 96.6.15

药量 μg/ml	OD值	抑制率%	IC50 μg/ml
1000	0.70 0.53 0.57 0.62	37	<0.98
	0.61 ± 0.07		
125	0.63 0.38 0.62 0.49	>65	
	0.53 ± 0.12		
250	0.50 0.49 0.64 0.87	39.3	
	0.63 ± 0.8		
125	0.47 0.43 0.53 0.66	>5.8	
	0.57 ± 0.11		
62.5	0.73 0.73 0.73 0.76	5.1	
	0.74 ± 0.02		
31.3	0.93 0.90 0.86 0.93	7.8	
	0.91 ± 0.03		
15.6	1.00 0.99 0.98 1.02	80.9	
	1.00 ± 0.02		
7.8	1.09 1.11 1.16 1.10	94.4	
	1.12 ± 0.03		
3.9	1.13 1.07 1.10 1.09	92.1	
	1.10 ± 0.03		
1.95	1.14 1.12 1.17 1.11	96.6	
	1.14 ± 0.03		
0.98	0.76 0.80 0.82 0.92	61.8	
	0.83 ± 0.07		
S.C	1.13 1.22 1.18 1.15		
	1.17 ± 0.04		

VCL605 0.24 0.29 0.31 0.26
0.28 ± 0.03

实验者：陈鸿珊 陈彩云
1996.6.20